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Overestimation of Glucose in Uranyl Acetate-Treated Samples: Modification of the Deproteinization Method

By *G. E. Hoffmann*,

Klinisch-Chemisches Institut, Städtisches Krankenhaus München-Harlaching,

K. Blum,

München and

L. Weiss

Klinisch-Chemisches Institut, Städtisches Krankenhaus München-Harlaching

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Summary: It is demonstrated that uranyl ions remaining in deproteinized supernatants of blood, serum, or liquor cerebrospinalis lead to falsely elevated glucose values by forming insoluble precipitates of uranyl phosphate. Furthermore, glucose is overestimated in hemolytic blood samples because of the UV-absorption of incompletely precipitated hemoglobin. A modified deproteinization method is recommended which overcomes these problems: the specimen volume is reduced to one half of the usual volume (0.05 instead of 0.1 ml) and excess uranyl ions are removed from the deproteinized supernatant by precipitation as uranyl phosphate, using phosphate-coated tubes. The supernatant obtained after centrifugation is free of uranyl ions and hemoglobin. Therefore, a sample blank may be omitted.

Überhöhte Glucosewerte in Uranylacetat-behandelten Proben: Modifikation der Enteiweißungsmethode

Zusammenfassung: Ein Überschuß an Uranylionen in enteiweißten Überständen von Blut, Serum oder Liquor führt durch Ausfällung unlöslichen Uranylphosphats bei der Glucosebestimmung zu falsch hohen Ergebnissen. Unvollständige Enteiweißung hämolytischer Blutproben verursacht auf grund der Eigenextinktion des Hämoglobins ebenfalls überhöhte Glucosewerte. Es wird deshalb eine modifizierte Enteiweißungsmethode vorgeschlagen. Das Probenvolumen wird auf die Hälfte des üblichen Volumens reduziert (0,05 statt 0,1 ml). In phosphat-beschichteten Gefäßen werden anschließend überschüssige Uranylionen als Uranylphosphat ausgefällt. Der so erhaltene Überstand ist frei von Hämoglobin und Uranylionen und kann ohne Berücksichtigung eines Probenleerwerts für die Glucosebestimmung verwendet werden.

Introduction

Uranyl acetate is widely used as a deproteinizing agent for blood glucose determination. It is effective at low concentrations and easier to handle than strong acids, e.g. perchloric acid. Polysaccharides are not hydrolyzed (1) and protein precipitates can be centrifuged at a relatively low speed (2). Recently, however, it was reported that uranyl acetate disturbs blood glucose determination at low sample protein concentrations (3).

Materials and Methods

All reagents were from Merck, Darmstadt, except Glucoquant and GOD-Perid test combinations, uranyl acetate solution, perchloric acid, and Precinorm S (Boehringer, Mannheim), bovine serum albumin (Behring, Marburg) Monitrol I–X, Monitrol II, and human hemoglobin standard (Merz and Dade, Miami), and Pathonorm low and high (Molter, Heidelberg).

Procedure

If not otherwise stated, deproteinization of blood, liquor cerebrospinalis, or control sera (0.1 ml each) was achieved by

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addition of 1 ml of either 0.33 mol/l perchloric acid or 3.8 mmol/l uranyl acetate. The precipitate was spun down within 2 min at 10 000 *g* (perchloric acid) or 3000 *g* (uranyl acetate). The supernatant was transferred to a plastic tube of the type used in the Eppendorf substrate analyzer (Substratautomat 5031) for glucose determination by the glucose dehydrogenase method (2). The tube was recentrifuged prior to assay (3000 *g*, 0.5 min) in order to remove the few erythrocytes which had been transferred together with the supernatant. For control experiments, the hexokinase (4) and glucose oxidase methods (1) were used. Protein and hemoglobin were determined by standard procedures (5,6).

Results and Discussion

Deproteinization of samples with low protein contents

Figure 1 demonstrates the poor agreement of glucose values determined after deproteinization by uranyl acetate and perchloric acid, respectively. Only at protein concentrations higher than 50 g/l do both methods yield identical results (fig. 2).

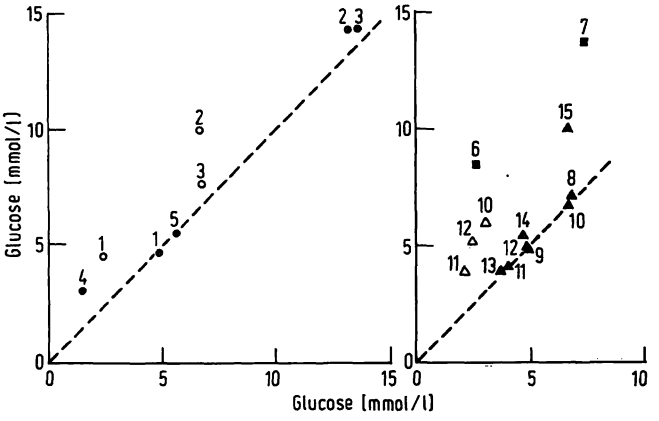

Fig. 1. Glucose concentrations measured in control sera (left) and biological fluids (right) after deproteinization by perchloric acid (abscissa) or uranyl acetate (ordinate), respectively. Filled symbols: undiluted samples. Open symbols: samples diluted 1:2. Numbered circles = control sera (1 = Monitrol I–X, 2 = Monitrol II, 3 = Pathonorm High, 4 = Pathonorm Low, 5 = Precinorm S), squares = liquor cerebrospinalis (6, 7), triangels = blood (8–15). The dotted line represents the graph of x = y.

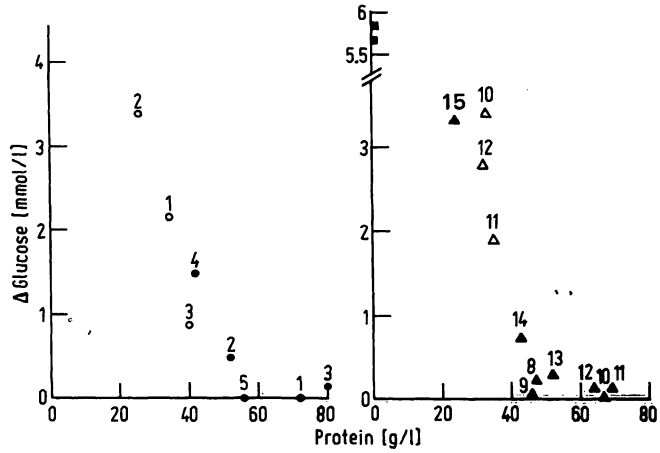

Fig. 2. Differences of glucose values (Δ glucose) determined in control sera (left) and biological fluids (right) after precipitation of varying amounts of protein by either perchloric acid or uranyl acetate. For symbols see figure 1.

It was demonstrated by measurements of the absorption spectra of uranyl acetate and deproteinized sample supernatants that at low protein concentrations an excess of uranyl ions remains in solution. Falsely elevated glucose values were proved to be the consequence of the turbidity caused by insoluble uranyl phosphate crystals (7), which were formed in the test tube in the presence of phosphate buffer. Precipitates were also obtained when the phosphate buffer was replaced by triethanolamine-HCl, Tris-HCl, or imidazole-HCl buffers, but not when triethanolamine-tartrate or imidazole-tartrate buffers were used. In the latter buffers, however, the activity of glucose dehydrogenase was markedly reduced as compared to phosphate buffer and, in addition, the uranyl tartrate complex formed (8) showed a very high absorption at 334 nm.

Using the hexokinase or glucose oxidase method, where phosphate buffer is also included in the test medium, the correct glucose content of a low protein serum (Pathonorm low) was measured (1.4 mmol/l), most probably because of the low sample volume added to the test volume. When reagent and sample volumes were the same as those used in the glucose dehydrogenase method (0.5 ml reagent + 0.05 ml deproteinized sample) and when the same incubation temperature (37 °C) and vigorous stirring procedure was applied, a 30–40 % overestimation of glucose was observed in the two control methods. The formation of uranyl phosphate was found to be enhanced by high temperature and stirring of the solution, conditions which are common to automated methods.

Complete removal of surplus uranyl ions from the deproteinized sample was achieved by precipitation of uranyl ions with phosphate prior to the glucose assay. 20 μmoles of sodium ammonium hydrogenphosphate (which is less hygroscopic than other soluble phosphates) were pipetted as a 1 mol/l solution into plastic tubes and dried at 40 °C overnight[1]).

Deproteinized samples were transferred to these phosphate-coated tubes, centrifuged as usual without further shaking and then analyzed in the Eppendorf substrate analyzer. Removal of uranyl ions by this procedure was sufficient, as indicated by the good agreement between glucose concentrations measured in these samples as compared to those deproteinized with perchloric acid (fig. 3).

Deproteinization of hemolytic blood samples

In hemolytic blood the protein concentration may be more than doubled by free hemoglobin. At a final concentration of about 4 mmol/l which is routinely used, the precipitating capacity of uranyl acetate is rather

[1]) Coated tubes are commercially available from Sarstedt, Nümbrecht, Germany.

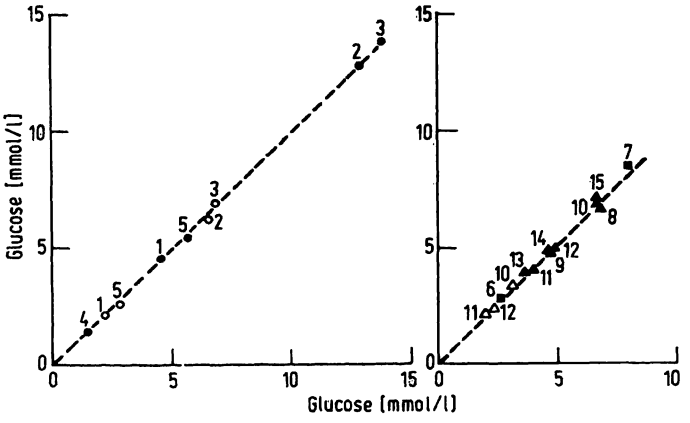

Fig. 3. Glucose concentrations measured in control sera (left) and biological fluids (right) after standard deproteinization by perchloric acid (abscissa) or by the modified uranyl acetate method (ordinate). For symbols see figure 1. Numbers 6–15 indicate identical specimens in all 3 figures.

Tab. 1. Precipitation of different proteins by uranyl acetate (0.1 ml protein solution + 1 ml uranyl acetate solution). The first column gives the protein concentration (bovine serum albumin, Monitrol I–x) or the hemoglobin concentration (human hemoglobin standard), respectively. Values are means of 2 determinations.

Protein concentration (g/l)	Percentage precipitated		
	Albumin	Hemoglobin	Monitrol
40	100	83	99
60	100	39	99
80	100	22	100
120	100	00	93
160	100	00	89

limited. It is shown in table 1 that even low amounts of hemoglobin are not completely precipitated under these conditions. Free hemoglobin leads to overestimation of glucose due to its absorption in the UV-range.

If, however, only 0.05 ml of hemolytic blood instead of 0.1 ml were added to 1 ml of uranyl acetate for deproteinization, hemoglobin precipitation was complete, despite total hemolysis. The excess of uranyl ions was sufficiently removed from the supernatant by phosphate precipitation as described above, so that glucose values corresponded well to those measured in non-hemolytic blood (tab. 2).

Considering the results of our study the following deproteinization method is recommended. 0.05 ml of whole blood, serum, or liquor cerebrospinalis are added to 1 ml of uranyl acetate. The sample is centrifuged for 2 min at 3 000 *g* and the clear supernatant is transferred to another tube containing about 20 µmol of dried sodium ammonium hydrogen-phosphate. After centrifugation (0.5 min at 3 000 *g*) the supernatant is used for glucose determination. This procedure warrants complete deproteinization of normal and hemolytic blood samples and removes excess uranyl acetate from the supernatant.

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Tab. 2. Glucose determination by the glucose dehydrogenase method (2) in 10 hemolytic blood specimens. Hemolysis was achieved by freezing twice in liquid nitrogen and subsequent thawing at 37 °C. 2 Deproteinization methods (0.1 ml blood and 0.05 ml blood, respectively, + 1 ml uranyl acetate) were compared. For removal of excess uranyl ions with phosphate see text. Reference values before and after hemolysis were obtained after deproteinization with perchloric acid.

Number of sample	Hemoglobin concentration (g/l)		Glucose concentration (mmol/l)			
	Total hemoglobin	Free hemoglobin after hemolysis	Before hemolysis	After hemolysis Deproteinization with		
				Perchloric acid	Uranyl acetate Routine method	Modified method
1	104	101	7.6	7.7	19.7	7.6
2	143	134	4.4	4.4	17.7	4.7
3	145	137	6.7	6.8	10.4	7.1
4	146	139	6.9	7.2	24.2	7.2
5	125	122	7.5	7.9	19.7	7.8
6	127	121	5.6	5.9	21.6	6.1
7	138	132	4.2	4.3	13.3	4.4
8	100	96	13.4	13.6	20.6	13.7
9	110	106	11.4	11.4	22.0	11.4
10	132	126	6.1	6.1	14.3	6.3

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Dr. med. Ludwig Weiss
Klinisch-Chemisches Institut
Städt. Krankenhaus München-Harlaching
Sanatoriumsplatz 2
D-8000 München 90